Aquatic fungi and fungus-like organisms isolated from surface waters situated near Białystok in Podlasie Province of Poland using the insect *Notonecta glauca* as bait

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Abstract. The growth of aquatic fungi and fungus-like organisms on the predatory heteropter *Notonecta glauca* was investigated in laboratory conditions. Water samples were collected from four sites varying in trophicity: Cypisek Spring, Biała River, Supraśl River, and Komosa Lake situated near Białystok. Certain physico-chemical parameters were determined in the samples. The highest contents of carbon dioxide, ammonium nitrogen, phosphates, and chlorides were noted in Biała River. Twenty-eight fungi and fungus-like organisms were isolated from *Notonecta glauca,* including 6 species of Chytridiales, 2 species of Blastocladiales, and 20 species of oomycetes. Fourteen fungi and fungus-like organisms were identified in Cypisek Spring, 8 in Biała River, 17 in Supraśl River, and 10 in Komosa Lake. Worthy of note is the finding of some aquatic fungi which have been commonly encountered on chitin-containing substrata, namely *Aphanomyces astaci, A. amfigynus, Chytriomyces hyalinus, Phlyctorhiza endogena, Podochytrium chitinophilum, Rhizidium chitinophilum,* and *Rh. nowakowskii.*

Key words: aquatic fungi and fungus-like organisms, Heteroptera, hydrochemical study, *Notonecta glauca,* Podlasie Province, Poland

Introduction

Chitinophilic fungi and fungus-like organisms growing on aquatic organic substrata, which fall to the bottom, accumulate chitin from animal remnants such as insect exuviae and wings. These fungi and fungus-like organisms play significant role in the aquatic ecosystems. They have marked proteolytic properties. Together with bacteria they are involved in decomposition of natural and hardly dissoluble polysaccharide, i.e. chitin that builds up the sarthropod skeleton. Fungi and fungus-like organisms mineralize chitin, thus contributing to the purification of the aquatic environment (Batko 1975; Söderhal & Dick 1991; Czeczuga & Godlewska 2001).

Notonecta glauca L. (Notonecticidae) is a sarthropod, relatively easily available to chitinophilic fungi. This is an eurhybiotopical species and its imagos were encountered throughout the year and inhabit diverse types of stagnant and running waters, such as mountain streams and limocreans. It is more common in moderately overgrown small and medium-size reservoirs. In rivers, it is found in marginal zones, mainly among vegetation (Krajewski 1969; Wróblewski 1980). It is also encountered in great numbers of swampy waters (Kurzątkowska 1993, 1999). It is a very active predator which feeds mainly on invertebrates such as shellfish, crustaceans, and insects or even vertebrates e.g. tadpoles, fish-fry and fish, often of bigger size than itself. It can be thus a fish parasite in fish farms. Water bugs are virulent animals, their bites could be very painful even for men (Pigulewski 1982; Engelhardt 1998).

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The main purpose of the study was to determine species composition of aquatic fungi and fungus-like organisms growing on the predatory insect *Notonecta glauca* in Cypisek Spring, Biała River, Supraśl River, and Komosa Lake situated in Białystok and its vicinity (Podlasie Province) as well as the effect of the ecological factors, including certain physical and chemical parameters of water and composition of the substratum from which the fungi were isolated.

Materials and Methods

The study on the occurrence of fungi and fungus-like organisms on the predatory aquatic insect *Notonecta glauca* was conducted in Cypisek Spring, Biała River, Supraśl River, and Komosa Lake.

1. Cypisek Spring is located at 53°21'N, 22°43' E in the south part of Knyszyński Forest, limnokrenic **type,** width 0.41 m, depth 0.17 m, discharge 0.6 l/s.
2. Biała River is located at 53°08' N, 23°09' E (length 9.8 km) is a left-bank tributary of middle part of the Supraśl River flowing trough Białystok City.
3. Supraśl River is located at 53°35'N, 22°52' E (length 106.6 km) is a right-bank tributary of the middle part of Narew River, flowing trough the Knyszyński Forest.
4. Komosa Lake is located 53°35'N, 22°52' E (12.1 ha, max depth 2.25 m) and is surrounded by extensive coniferous woods of Knyszyński Forest.

Eight water samples were collected from each site. Bait method using fragments of *Notonecta glauca* introduced to water samples from each reservoir was employed to isolate fungi and fungus-like organisms.

The aquatic insects used for analysis were collected in old river-beds of the Niemen near Orlja in Belorussia, in high peatbogs of Galwica near Olsztyn, from Kortowskie Lake and a small down-town reservoir in Olsztyn of Poland.

Fragments of the insects were placed in one-litre containers with water samples collected from the respective reservoir or watercourse. The containers were covered with glass plates to protect the water, at least partly, against bacteria. The samples were stored for four weeks in the laboratory at a temperature approximating that of water in the springs, rivers, ponds and lakes in the respective month. At that time, lighting and warming were regulated. Microscopically identified mycelia were transferred to sterilized Petry plates containing distilled water. The affected insects were observed under an optic microscope 100× and 400× every day starting from the third day of the culture. At the same time the respective developmental stages of fungi were determined using an ocular micrometer. The identification of fungi and fungus-like organisms involved such morphological features as shape and size of hyphae, shape of sporangium and spores, and the structure of oogonium, oosporum, and antheridium. The fungi and fungus-like organisms were identified using the works of Bedenek (1972), Batko (1975), Dick (1990), and Pystina (1998).

Water samples used for physico-chemical analysis were collected approximately 0.20 m from under the water surface with a Ruttner's apparatus of 2.0 dm^3 vol. In natural conditions and in the laboratory, the hydrochemical analysis was performed to determine temperature, pH, oxidability, carbon dioxide, total alkalinity, sulphates, ammonium nitrogen, nitrite nitrogen, nitrate nitrogen, phosphates, total iron, chlorides, calcium, magnesium, dry residue, dissolved, and suspended substances. Physico-chemical investigations were performed using commonly applied hydrobiological methods used in Poland (Dojlido 1995).

Results

The physico-chemical analysis of water, used for the experiment, showed that the river Biała was the most abundant in carbon dioxide, ammonium nitrogen, phosphates, and chlorides in comparison to the other water bodies. Biała River and Supraśl River have the highest oxidability (Tab. 1).

The common water bug (*Notonecta glauca*) was the habitat for 28 species of fungi and fungus-like organisms, including 8 of Chytridiomycetes, and 20 of Oomycetes (Tab. 2, Figs 1-5). 14 species were isolated from Cypisek Spring, 8 from Biała River, 17 from Supraśl River, and 10 from Komosa Lake. Worthy of note is the finding of fungi and fungus-like organisms which occur on chitin-containing substrata, such as *Aphanomyces astaci, A. amfigynus, Chytriomyces hyalinus, Phlyctorhiza endogena, Podochytrium chitinophilum, Rhizidium chitinophilum,* and *Rh. nowakowskii.*

Discussion

Among the fungi and fungus-like organisms found on the predatory insect *Notonecta glauca,* the following species are worthy of note: *Aphanomyces astaci, A. amfigynus, Chytriomyces hyalinus, Phlyctorhiza endogena, Podochytrium chitinophilum, Rhizidium chitinophilum,* and *Rh. nowakowskii.*

Besides the eight species of chytridia isolated in the study, *Rhizidium chitinophilum* found in Supraśl River and Komosa Lake, was the most frequently encountered fungus. Karling (1967b) observed *Rh. chitinophilum* in soil samples in New Zealand on chitin carapaces of crustaceans – shrimps and insect larvae, it was also identified by Czeczuga *et al.* (1984) on insect exuviae in the river Narew and by Czeczuga (1991) in Wigry Lake.

Phlyctorhiza endogena and *Podochytrium chitinophilum* were found on this water bug in Cypisek Spring. These fungi have also been isolated from termite wings in lakes in New Zealand (Karling 1967a) and dragon-flies and flies in trophically varied water reservoirs of Podlasie Province (Czeczuga & Godlewska 1998).

Blastocladiella britanica was found in Supraśl River. In Poland it was also isolated from Biebrza River (Czeczuga *et al.* 1990) and from Wigry Lake (Czeczuga 1991). According to

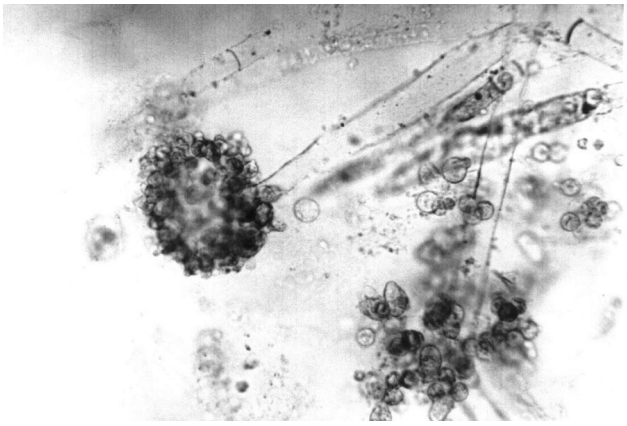

Fig. 1. *Achlya klebsiana* – hyphae from sporangium

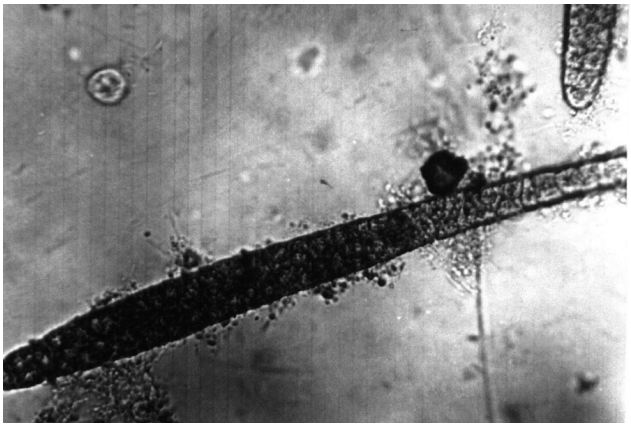

Fig. 2. *Dictyuchus monosporus* – hyphae from sporangium

Fig. 3. *Leptolegnia caudata* – hyphae from sporangium

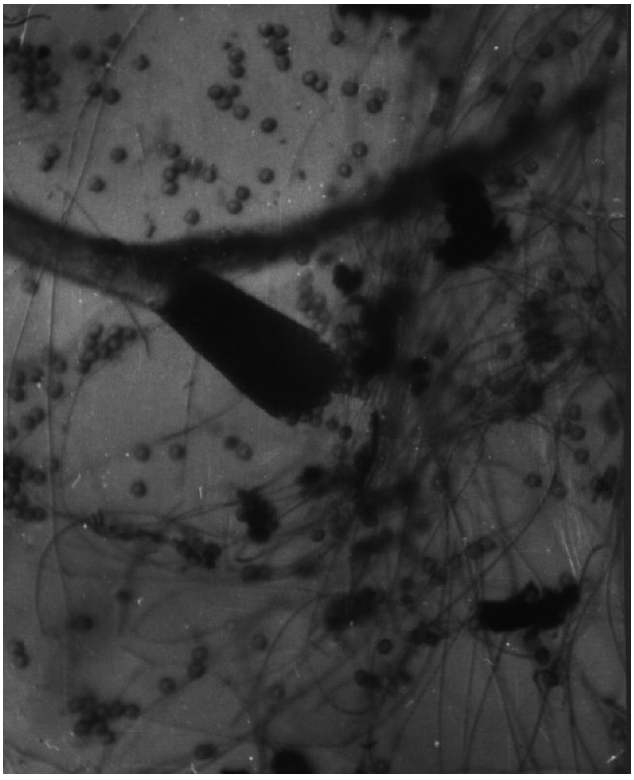

Fig. 4. *Thraustotheca clavata* – sporangia

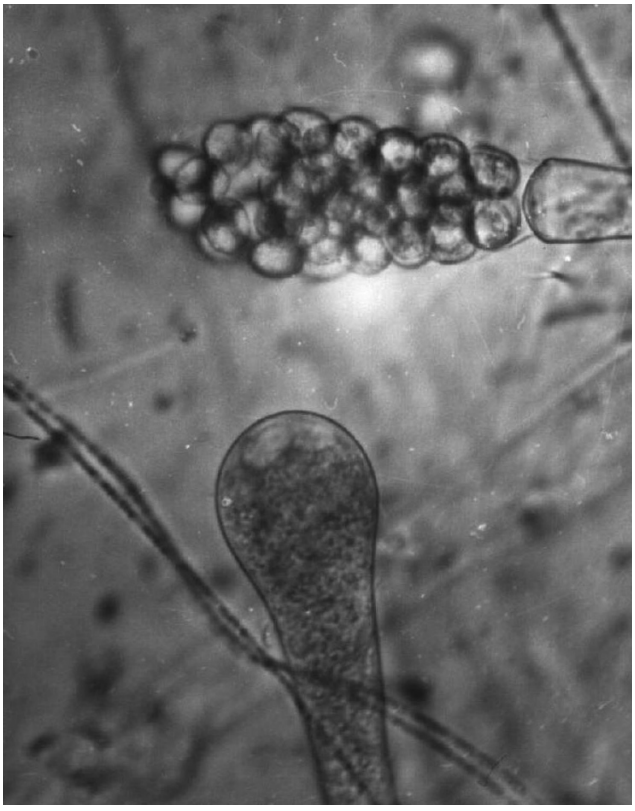

Fig. 5. *Thraustotheca clavata* – sporangia

Table 1. Physico-chemical parameters (in mg l^{-1}) of water from the different sites (mean from 6 samples)

Specification	Cypisek Spring	Biała River	Supraśl River	Komosa Lake
Temperature (°C)	9.2	12.0	11.5	13.5
pH	7.00	7.49	7.09	7.16
Oxidability	4.6	10.36	12.05	9.10
CO_2	24.20	52.48	8.80	17.60
Alkalinity (mval l^{-1})	5.7	4.4	4.3	4.5
$N\text{-}NH_4$	0.51	2.24	0.25	0.25
$N\text{-}NO_2$	0.006	0.031	0.011	0.140
$N\text{-}NO_3$	1.484	0.270	0.011	0.003
$P\text{-}PO_4$	0.134	1.220	0.170	0.045
Chlorides	30.20	78.94	32.00	25.00
Total hardness in Ca	131.10	105.10	72.00	77.76
Total hardness in Mg	26.73	20.65	20.64	17.63
$S\text{-}SO_4$	63.80	54.20	22.21	41.96
Fe	0.00	0.56	0.45	0.30
Dry residue	520	470	305	363
Dissolved solids	479	451	265	270
Suspended solids	41	19	40	93

Horenstein & Cantino (1961), the formation of sporangium and spores is determined by light conditions – the fungus develops faster in light then in darkness. *Blastocladiopsis parva* was found in the two rivers involved in the study. This fungus, known as a phyto- and zoosaprobe was previously isolated from chitin and keratin-containing substrata, on crayfish (Czeczuga & Godlewska 1994; Czeczuga *et al*. 1998), on fish in Jaroszówka Spring and in the pond Akcent in Białystok (Czeczuga *et al.* 2002b) and on seeds and a snake skin in Biebrza River (Czeczuga *et al*. 2003).

Representatives of the class Oomycetes were the most numerous group of fungus-like organisms found on the predatory insect *Notonecta glauca.* Twenty taxa were isolated. *Achlya americana* was isolated from the water bug in Cypisek Spring and Komosa Lake. Khulbe & Sati (1981) found this species on fish living in Asian mountain rivers. In Poland, *A. americana* was observed on crayfish (Czeczuga *et al*. 1998). *A. debaryana,* frequently found on *Notonecta glauca,* was isolated from Cypisek Spring and Supraśl River. Czeczuga (1993) isolated this oomycete from high peatbogs of Gorbacz and Ostrówek. This is an aquatic-soil saprobe, growing like *A. americana* in the water on tree branches and insects (Batko 1975). It should be noted that one of the sites from which insects were collected for analysis was high peatbog in Galwica near Olsztyn. *A. klebsiana* was found only in Cypisek Spring. It was isolated by other authors on capped mushrooms, membranous wings of fly, and cover wings of potato beetle in ponds in Białystok (Czeczuga & Godlewska 1994). Interesting is the finding of the fungus-like organism *Leptolegnia caudata* on *Notonecta glauca* in Cypisek Spring. This species was encountered as a parasite of predatory crustaceans *Leptodora kindtii* (Batko 1975). *Leptolegnia caudata* was observed to be an aquatic and soil saprobe found on crustaceans, dragonflies, snake skin, and spawn of various fish species (Dudka *et al.* 1989; Czeczuga & Muszyńska 1994; Czeczuga *et al*. 2002d).

Saprolegnia ferax and *S. parasitica* were frequently isolated taxa throughout the experiment. These oomycetes are commonly encountered both on dead vegetation and animal material. However, in favourable conditions they are parasites on weakened and mechanically damaged animals, mainly spawn and fish in their different developmental periods (Hatai & Hoshiai 1992a, b; Czeczuga *et al.* 1995).

Thrausthoteca clavata, an aquatic-soil saprobe, was found on *Notonecta glauca* in Cypisek Spring. It was frequently encountered in inland waters (Dudka *et al.* 1989; Czeczuga *et al.* 2002c).

Species of the genus *Pythium,* such as *P. elongatum, P. gibbosum, P. jirovecii, P. myriotylum,* and *P. rostratum* were also regularly found on common water bugs in water samples from the respective sites. Until recently, these species were regarded as aquatic and soil saprobes or plant parasites (El-Nagdy 1991). Later studies have revealed that *Pythium* species grow in water reservoirs of varied trophicity (springs,

Table 2. Fungi and fungus-like organisms isolated from surface waters in Podlasie Province using the insect *Notonecta glauca* as bait

Taxa	Cypisek Spring	Biała River	Supraśl River	Komosa Lake
FUNGI				
Chytridiomycetes				
Chytridiales				
**Chytriomyces hyalinus* Karling				×
Chytridium xylophilum Cornu				×
**Phlyctorhiza endogena* Hanson	×			
**Podochytrium chitinophilum* Willoughby				×
**Rhizidium chitinophilum* Sparrow			×	×
**Rhizidium nowakowskii* Karling			×	
Blastocladiales				
**Blastocladiella britannica* Horenstein & Cantino			×	
Blastocladiopsis parva (Whiffen) Sparrow		×	×	
CHROMISTA				
Oomycetes				
Saprolegniales				
Achlya americana Humphrey	×			×
Achlya crenata Ziegler	×			
Achlya debaryana Humphrey	×	×		×
Achlya klebsiana Pieters	×			
**Aphanomyces astaci* Schikora	×	×		
Aphanomyces irregularis Scott	×	×	×	
Aphanomyces laevis de Bary		×	×	×
**Aphanomyces amfigynus* Cutter	×		×	×
Dictyuchus monosporus Leitgeb			×	
Leptolegnia caudata de Bary	×			
Saprolegnia ferax (Gruith.) Thur.	×	×	×	×
Saprolegnia parasitica Coker	×	×	×	×
Saprolegnia unispora (Coker & Couch) R.L. Seym.			×	
Thraustotheca clavata (de Bary) Humphrey	×			
Pythiales				
Lagenidium marchalianum de Wildeman	×		×	
Pythium elongatum Matthews			×	
Pythium gibbosum de Wildeman			×	
Pythium jirovecii Cejp			×	
Pythium myriotylum Drechsler			×	
Pythium rostratum E.J. Butler	×	×	×	
Total number	**14**	**8**	**17**	**10**

* species found on a chitin substratum

rivers, ponds, and lakes) on vegetation, dead animals or they can also be animal parasites. Oomycetes of this genus have caused losses in the breeding of shrimps, crabs, and fish (Suzuki & Hatekayama 1960; Hendrix & Campbell 1969; Mescheryakova & Logvinienko 1970; Park & McKee 1978; Park 1980, Abdelzahler *et al.* 1994a, b; Czeczuga 1996).

The predatory aquatic insect *Notonecta glauca* constitutes a good substratum for the isolation of fungi and fungus-like organisms from water, especially such chitinophilic species as: *Aphanomyces amfigynus, A. astaci, Chytriomyces hyalinus, Phlyctorhiza endogena, Podochytrium chitinophilum, Rhizidium chitinophilum,* and *Rh. nowakowskii.* Unestam (1996), studied saprobic and parasitic fungi and fungus-like organisms and showed that they produce an enzyme – chitinase. In his observation of chitinophilic, cellulophilic, and pectinophilic activity of such species as *Aphanomyces, Saprolegnia,* and *Pythium,* the author found that they all produced chitin-decomposing chitinase. In the present study, of the seven species it was *Aphanomyces astaci* that had the highest chitinophilic activity. Söderhäl *et al.* (1978) confirmed chitinophilic properties of this fungus-like organisms isolated from crayfish. *A. astaci* causes crayfish plague, which leads to death (Unestam 1965). It was found on *Notonecta glauca* only in Cypisek Spring. Its growth was also observed on plankton and benthos crustaceans (Czeczuga *et al.* 1998, 1999, 2000, 2002a), on aquatic insects (Czeczuga & Godlewska 2001), and on arachnids (Kiziewicz & Czeczuga 2003).

The physico-chemical analysis of water, used for the experiment, revealed the highest content of carbon dioxide, ammonium nitrogen, and phosphates in the river Biała in comparison to the other reservoirs. Biała River and Supraśl River have the highest oxidability. In heavily polluted reservoirs, characterized by high oxidability and high content of biogenes, the species composition of fungi and fungus-like organisms varies more than in less polluted waters. However, when the content of pollutants exceeds the range of tolerance for the respective species of fungi and fungus like organisms, it delimits their occurrence (Dojlido 1995). This is probably the reason why the fewest fungi and fungus-like organisms were found to grow on *Notonecta glauca* in the river Biała, which was eutrophic.

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